JOURNAL OF BACTERIOLOGY, Sept. 1979, p. 1079-1081
0021-9193/79/09-1079/03$02.00/0

Vol. 139, No. 3

Effect of Transcription on RecBC- and RecF-Mediated Recombination Within the Tryptophan Operon of *Escherichia coli* K-12

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Received for publication 13 June 1979

Recombination between tryptophan gene mutations within the *trp* operon was determined among transductants for an outside linked *cysB* marker under conditions of repression and derepression. These studies, carried out with recipient strains utilizing the RecBC or RecF pathway, or a combination of these pathways of recombination, demonstrate that transcription of *trp* genes as regulated by the *trp* repressor has no significant effect on RecBC- or RecF-mediated recombination within the *trp* operon.

Conflicting reports have emerged from studies of the effect of transcription on recombination. Transcription of the *ara* operon stimulates recombination within that operon by about 50% (4). Induction of genes involved in the metabolism of lactose has been found to inhibit by about one-half the frequency of recombination between the chromosome and an F′ episome harboring the *lac* operon (5) or to have no effect on recombination of conjugational zygotes of the *lac* operon (10). The efficiency of transduction by (λ-*gal*)T1 is increased by the transcription of the phage genome (3). Recently it was reported that illegitimate recombination requires transcription (6, 9).

It was of interest to determine if transcription of the *trp* operon genes involved in tryptophan biosynthesis had an effect on recombination within that operon. Since *Escherichia coli* has more than one pathway of recombination available to it (1, 2), it was also of interest to learn whether transcription would affect recombination differently in strains which use the RecBC or RecF pathway, or a combination of the two, to carry out recombination.

To study this question, three sets of isogenic recipient strains were constructed, each bearing a common *cysB38* mutant allele and one of three different mutant *trp* alleles (see Table 1) plus mutations in the *recB recC* and/or *sbcB* genes. Thus, each set of strains is composed of strains which mediate recombination via the RecF pathway (*recB recC sbcB*), the RecBC pathway (*recB*$^+$ *recC*$^+$ *sbcB*$^+$), or both of these pathways (*recB*$^+$ *recC*$^+$ *sbcB*). Strain backgrounds and constructions are described in Table 1 and elsewhere (manuscript submitted for publication). In addition, each strain was constructed in two forms (*trpR*$^+$ and *trpR*) so as to create a series of isogenic pairs of strains differing only with respect to the state of the tryptophan regulator gene, *trpR*.

These strains were used as recipients in transductions with a P1 phage lysate grown on a bacterial donor strain bearing a mutation in the *trpA* gene but wild type for *cysB*. Cys$^+$ transductants were selected on minimal medium lacking cysteine but containing tryptophan and were then screened by replica-plating for the Trp$^+$ character. The tryptophan in the medium repressed transcription of the *trp* operon in *trpR*$^+$ strains, whereas the *trpR* strains remained derepressed (8). Since the *trpA* mutation in the donor is distal to all of the three *trp* mutations in the various recipients, any Trp$^+$ colony would have to arise because of a cross-over event occurring within the *trp* operon (see Fig. 1). A comparison of the fraction of Cys$^+$ colonies that were also Trp$^+$ in strains that are *trpR*$^+$ and *trpR* should demonstrate whether transcription of *trp* genes has any effect on recombination within that operon. Since these crosses were also done in recipients which differ with respect to their Rec pathways, such an experiment can disclose whether transcription effects (if any) upon recombination differ in the several pathways of recombination that appear to occur in *E. coli*.

Table 1 shows the results of these crosses. The fraction of Trp$^+$ Cys$^+$ colonies among Cys$^+$ colonies increases, as expected, with increasing distance of the recipient's *trp* marker from the donor's *trp* marker. However, there is no significant difference between the data obtained from the *trpR* and *trpR*$^+$ versions of each strain. A mutation in the *trpR* gene results in a 70-fold

TABLE 1. *Recombination within the trp operon under conditions of repression and derepression with isogenic recipient strains which differ with respect to their Rec pathways*

Recipient strain[a]	Recipient alleles					Recipient Rec pathway	% Trp$^+$ recombinants[b] ± standard error
	trp	*trpR*	*recB*	*recC*	*sbcB*		
SC236	E10220	+	−	−	−	RecF	12.7 ± 0.7 (2,487)[c]
SC239	E10220	−	−	−	−	RecF	11.3 ± 0.9 (1,188)
SC235	E10220	+	+	+	−	RecF, RecBC	19.7 ± 0.7 (3,432)
SC237	E10220	−	+	+	−	RecF, RecBC	17.7 ± 1.1 (1,305)
SC234	E10220	+	+	+	+	RecBC	13.0 ± 0.7 (2,487)
SC238	E10220	−	+	+	+	RecBC	13.7 ± 0.9 (1,542)
SC216	D562	+	−	−	−	RecF	8.7 ± 0.8 (1,355)
SC219	D562	−	−	−	−	RecF	8.7 ± 0.8 (1,326)
SC231	D562	+	+	+	−	RecF, RecBC	13.0 ± 1.0 (1,129)
SC232	D562	−	+	+	−	RecF, RecBC	10.7 ± 0.9 (1,071)
SC218	D562	+	+	+	+	RecBC	6.1 ± 0.7 (1,088)
SC221	D562	−	+	+	+	RecBC	6.2 ± 0.7 (1,159)
SC225	C782	+	−	−	−	RecF	7.5 ± 0.8 (972)
SC228	C782	−	−	−	−	RecF	7.3 ± 0.7 (1,324)
SC224	C782	+	+	+	−	RecF, RecBC	10.1 ± 1.0 (975)
SC227	C782	−	+	+	−	RecF, RecBC	9.1 ± 0.7 (1,816)
SC226	C782	+	+	+	+	RecBC	6.1 ± 0.7 (1,210)
SC229	C782	−	+	+	+	RecBC	4.4 ± 0.6 (1,296)

[a] In addition to the *cysB* and *trp* mutations shown on the table, these strains have the following markers: *his-4 ara-14 thr-1 thi-1 lacY-1 mtl-1 xyl-5 galK2 proA2 argE3 str-31 tsx-33 sup38*(Am). Isogenic strains which differ with respect to their Rec pathways were constructed by interrupted matings of *thy* derivatives of SC236, SC216, or SC225 with HfrKL16. SC236, SC216, and SC225 were formed by selecting *thy*$^+$ *recB recC* strains from their respective *thy* derivatives. SC235, SC231, and SC224 were formed by selecting *thy*$^+$ *recB*$^+$ *recC*$^+$ strains and SC234, SC218, and SC226 were constructed by selecting *thy*$^+$ *recB*$^+$ *recC*$^+$ *his*$^+$ *sbcB*$^+$ strains. All *trpR* strains were constructed by transducing the *trpR*$^+$ parent with P1 phage grown on a donor harboring *thr*$^+$ *trpR41* alleles, selecting for Thr$^+$ recombinants, and screening for strains resistant to 100 μg of 5-methyltryptophan per ml.

[b] All recipient strains carried the *cysB38* mutant allele causing cysteine auxotrophy plus the mutant *trp* alleles as shown above, whereas all donor strains bore the *cysB*$^+$ allele and the mutant *trpA9952* allele. In all crosses *cysB*$^+$ was the selected marker, and the presence of tryptophan in the agar made the state of the *trp* genes an unselected marker. The frequencies of Trp$^+$ recombinant clones were determined by replica-plating and are expressed as a percentage of the total number of Cys$^+$ transductants examined.

[c] Numbers in parentheses show total number of transductants tested for each cross. The totals were usually obtained by summing results from several repeats of each cross.

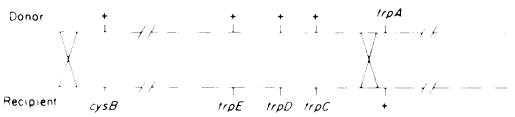

FIG. 1. *Diagram of example of breakage and reunion regions between donor and recipient chromosomes which would result in a Cys*$^+$ *Trp*$^+$ *recombinant. The point mutation positions are not drawn to scale but represent approximately correct relative distances from each other, based on data reported elsewhere (7).*

increase in the synthesis of the operator-proximal genes, *trpE* and *trpD*, and about a 20-fold increase in the synthesis of the operator-distal genes, *trpC*, *trpB*, and *trpA*, over that found in fully repressed cells (7). The derepression ratio (R^-/R^+) differs for operator-proximal and operator-distal genes because an internal low-efficiency promoter located near the distal end of gene *trpD* results in low-level constitutive synthesis of gene products downstream from it even under conditions of repression (7). The results presented here demonstrate that there appears to be no effect of repression or derepression of transcription of the *trp* operon upon the frequency of recombination within that operon. Table 1, however, demonstrates that a higher frequency of Trp$^+$ colonies occurs among Cys$^+$ transductants in strains that utilize a combination of RecF and RecBC recombination pathways as compared with that observed with strains having only a functional RecF or RecBC pathway. This finding suggests that the nature of recombinants formed in genetic crosses may depend upon the recombination pathway used by the cell. Such data will be presented in greater detail in another paper (submitted for publication).

The results described above demonstrate that transcription of the *trp* genes as regulated by its primary means of regulation, the *trp* repressor,

has no significant effect on RecBC- or RecF-mediated recombination within the *trp* operon of *E. coli*.

I thank M. Oishi and C. Yanofsky for supplying strains, T. Fuchs for excellent technical assistance, and P. Margolin for helpful suggestions in the preparation of this manuscript.

This investigation was supported by Public Health Service research grant GM22562 from the National Institute of General Medical Sciences and in part by a grant from the City University of New York PSC-BHE Research Award Program (RF11396).

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